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SOME REMARKS UPON THE PUBLICATION OF PRESTON KYES ENTITLED "VENOM HEMOLYSIS."*

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In Vol. 7, No. 2, March, 1910, of this journal, Kyes has presented a summary of his earlier work on the subject of venom hemolysis with some new experiments, most of which are of the nature of repetitions. To him belongs the credit of recognizing that the snake venom hemolysis is brought about by the formation from lecithin, through the action of the venom, of the true hemolysin.

That the split products of lecithin should be hemolytic is, indeed, not extraordinary, but Kyes believes that in the reaction between cobra venom and lecithin something of a quite extraordinary nature takes place for which no analogy can be found either in chemistry or in the field of serology. The cobra venom is supposed to unite with the "lecithin-rest," and on the one hand to be a chemically definable substance, and on the other to possess the nature of the chemically indefinable toxins, inasmuch as it can give rise, in the animal body, to the production of antitoxin. The chemists who undertook the analysis of the new substance could, it is true, find only the lecithin-rest, while the toxins still defied chemical analytical methods. The only support, therefore, for an assumed toxine nature lay in the alleged property of the substance to stimulate the production of antitoxin.

In our previous publications[1] we were able to show that Kyes's view is incorrect. Antibodies cannot be produced against the so-called "lecithid," that is, against the derivative of lecithin which produces immediate hemolysis without incubation period. The experimental conditions under which Kyes worked were complicated

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[1] *Münch. Med. Wchnschr.*, 1908, 55, p. 105; *Biochem. Ztschr.*, 1908, 12, p. 407.

by the presence, in his preparation of "lecithid," of the hemolysin of native cobra venom, *and it was this, which upon injection into animals, caused the production of antitoxin, and against which alone the action of the antitoxin could be demonstrated.* The proof which we adduced against the toxin nature of the lecithin hemolysin is so convincing that further evidence would be entirely superfluous. Ehrlich and Sachs have also accepted our view and Sachs himself was never able to produce antitoxin by the injection of *heated* "lecithid."

In this connection, and in view of the statement of Kyes that the "lecithid" used by him for his immunization experiments had been boiled, it should be said that occasionally preparations of the lecithin derivative (which we have designated as "desoleolecithin") are obtained by Kyes's method in which, for some unknown reason, the native cobra hemolysin is resistant even to boiling.

EXPERIMENT.

From a 1 per cent watery solution of "desoleolecithin," prepared three weeks previously with freshly made ovo-lecithin, a further dilution 1–10 was made in physiological salt solution representing a final 1–1,000 concentration. A portion of this was used as control, a second portion boiled for one minute, and a third portion also boiled for one minute after the addition of an equal volume of $\frac{n}{10}$ acetic acid. After neutralization of the third portion and equalization of the volume in the three portions, it was found that the hemolytic power against washed ox corpuscles without the addition of lecithin was identical in all three; namely, $\frac{1}{60}$ c.c. of a 1–1,000 concentration.

With the addition of 0.5 c.c. of a 1–2,000 suspension of ovo-lecithin (1.0 c.c. of this suspension by itself was not hemolytic) the hemolytic power was equally increased in *all three portions* to $\frac{1}{240}$ c.c. of a 1–1,000 concentration, $\frac{1}{320}$ c.c. producing nearly complete hemolysis. These results are identical with those obtained by the examination made three weeks previously, during which period the preparation had been kept in the ice-box.

It is surprising, therefore, that Kyes, although he has not refuted our criticisms of his experiments, still clings to his disproven view. The new results which he presents in favor of the antigenic nature of "lecithid" are obtained by the very methods which we have shown to be fallacious. He again uses the antiserum after heating it to 64°–65° C., which seems to indicate that, without heating, the inhibiting effect could not be observed. That Kyes is in error, if he still believes that his experiments are not controverted by our own, must be evident from the following citation:

> "We convinced ourselves first of the correctness of Kyes's statement that a distinct difference is shown in the action of immune and normal sera when these have been heated for one-half hour at 64° C. It was found that heated normal rabbit's sera influenced the hemolytic activity of the lecithin derivative preparation in different degree, but that the inhibitory effect of the immune sera was still much more marked. The result was quite different, however, when 'lecithid,' serum, and blood were mixed at one time, and only that hemolysis taken into account which occurred within a few minutes. Under these conditions the heated immune sera protected exactly as did the heated normal sera. Since the lecithin derivative, in contrast with the native venom combined with lecithin, dissolves the corpuscles very quickly, and this is true of the smallest hemolytic dose, the result just described was very remarkable. It indicated that the specific antitoxin which was present in the immune sera but not in the normal sera was not directed at all against the lecithin derivative. Other circumstances also favored this view. It was further observed that the late hemolysis took place only on condition that *heated* normal serum was added. Fresh unheated serum did not impede the hemolysis at all. It was also noticed that the unheated immune sera did not cause any greater inhibition than normal sera and that the heated normal sera were exactly as antihemolytic as the unheated sera provided only that the quickly occurring hemolysis, which is characteristic of the action of the lecithin derivative, was taken into consideration. All of these observations were incompatible with Kyes's assumption that normal antitoxin is destroyed by heating. They showed very clearly that the heating did not eliminate an inhibiting property of the serum but gave to the serum a new quality—one that permitted hemolysis. The significance of this secondary hemolysis was easily recognized in its long period of incubation, which pointed clearly to a combination of lecithin with native cobra-venom. In the heated serum lecithin was present in *available* form, in which form it is not necessarily present in unheated serum; moreover, in the preparations of the lecithin derivative obtained by Kyes's method the active hemolytic constituent of the venom could quite conceivably be contained in solution. Our further experiments have established the correctness of this explanation in several ways.
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> "In the first place we were able to reproduce all the phenomena that accompany the use of heated sera by the addition of lecithin. Under this condition the same difference between normal and immune sera is made manifest.
>
> "We found, furthermore, that, in the 'lecithid' preparation which we were using, the native venom constituent which is capable of acting upon lecithin was present in considerable quantity. Such small quantities of the preparation as were by themselves incapable of causing any solution of the corpuscles could produce complete hemolysis, after a period of incubation, upon the addition of lecithin."

In our second publication[1] we could demonstrate in this way about 40 per cent of the native hemolytic constituent unchanged in the chloroform lecithin layer, and in a recent experiment the freshly prepared "desoleolecithin" was found to contain as much as 60–70 per cent of the active ferment.

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[1] *Loc. cit.*

> "Finally, by heating the preparation in 1 per cent solution for three hours at 100° C. we succeeded in so altering it that it could no longer be 'activated' by the addition of lecithin. Under this treatment the rapid hemolytic power of the substance remained unchanged. By this means we obtained a 'lecithid,' the hemolytic activity of which was inhibited equally by immune and normal sera even when these were heated at 64° C. against this preparation. Also the inhibition of the heated hemolysin by heated normal sera corresponded with the inhibition of the unheated hemolysin by the unheated normal and immune sera. Every difference between the normal and the immune sera had disappeared.
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> "From all these facts it is evident that by the immunization antitoxin was produced, not against the lecithin derivative, but only against the native venom constituent contained in it."

Since the newer experiments of Kyes contain the same sources of error, the results are no more available as argument in this discussion than his earlier ones.

It is remarkable that Kyes pays no attention to this point upon which the entire argument rests, but contents himself with a criticism of the method with which we prepared the "desoleo-lecithin." We are satisfied that no importance attaches to the method of preparation, yet we would call attention to the fact that where the addition of alkali seemed necessary we have made it, and furthermore that the immunization of our rabbits was carried out with a preparation which had been made with the addition of alkali—as is specially mentioned in our publication.

We do not see the least reason in Kyes's article for changing our view, a further discussion of which is rendered superfluous by the more recent study of Manwaring,[1] who confirmed our results in every detail.

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[1] *Ztschr. f. Immunitätsf.*, 1910, 6, p. 513.